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Effect of the nootropic drug oxiracetam on field potentials of rat hippocampal slices

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1 The effect of the nootropic drug oxiracetam on hippocampal neurotransmission was investigated in the CA1 region of the rat hippocampal slice *in vitro* by use of extracellular recordings.

2 Superfusion of oxiracetam (0.1–100 μM) produced a concentration-dependent, wash-resistant (>90 min), increase in initial slope and amplitude of the dendritic field excitatory postsynaptic potential (e.p.s.p.). This increase was maximal at a concentration of 1 μM (70%).

3 Input-output curves relating the initial slope to the amplitude of the afferent volley were significantly ($P < 0.05$) steeper and showed a greater maximal response in the presence of 1 μM oxiracetam than in control conditions.

4 Two trains of high frequency stimulation (100 Hz, 0.4 s, 5 min apart) delivered in the stratum radiatum 30 min after washout of oxiracetam (1 μM) still elicited a long-term potentiation (LTP) of the field e.p.s.p. However, the absolute magnitude of the LTP produced did not differ from that obtained in untreated slices.

5 After induction and establishment of LTP, oxiracetam (1 μM) had a smaller (27%) and reversible effect on the evoked field e.p.s.p.

6 D-2-Amino-5-phosphonopentanoic acid (AP-5), at the same concentration (50 μM) which in our conditions prevented the induction of LTP, blocked the action of 1 μM oxiracetam and strongly depressed the effect of higher concentrations of the nootropic drug.

7 It is concluded that oxiracetam provokes an enduring increase of neurotransmission in the CA1 rat hippocampal region. This action appears to share some features with LTP as indicated by its persistence, sensitivity to AP-5 and lack of additivity with electrically-induced LTP.

Introduction

Oxiracetam (4-hydroxy-2-oxopyrrolidinoacetamide) counteracts the severe cognitive impairment associated with methylazomethanol-induced microencephalia in rats (Banfi *et al.*, 1984) and antagonizes the amnesic effect brought about by hypoxia, cycloheximide (Banfi & Dorigotti, 1986), electroconvulsive treatment (Spignoli & Pepeu, 1986) and scopolamine (Spignoli & Pepeu, 1987) in mice and rats. The clinical effects of oxiracetam on cognitive processes have repeatedly been demonstrated (Gainotti *et al.*, 1986; Giaquinto *et al.*, 1986). The pharmacological actions of oxiracetam are similar to those described for other pyrrolidone derivatives, including piracetam, aniracetam, pramiracetam (Moos *et al.*, 1988; Pepeu & Spignoli, 1989) which are commonly defined as nootropics. According to Giurgea & Salama (1977) and Schindler *et al.* (1984) the term nootropic agent indicates a drug which facilitates learning and memory and prevents the impairment of cognitive functions induced by brain insults and disease.

It is commonly believed that hippocampal long-term potentiation (LTP) provides a model for a cellular mechanism related to learning and memory (Teyler & DiScenna, 1987; Nicoll *et al.*, 1988; Brown *et al.*, 1988). It may therefore be expected that nootropic drugs influence hippocampal LTP. An increase in the amplitude of hippocampal population spike has been observed by Olpe & Lynch (1982) in the CA1 region after application of piracetam. Satoh *et al.* (1986, 1988) have demonstrated that in the CA3 region of the guinea-pig hippocampal slice, aniracetam and piracetam enhance LTP. Such an effect is characterized by a bell-shaped concentration-response curve.

In the present study on rat hippocampal slices the effects of oxiracetam on dendritic responses evoked in CA1 pyramidal cells by electrical stimulation of stratum radiatum were investigated in an attempt to clarify its receptor mechanisms. A preliminary account of some of the results was given at a meeting of the British Pharmacological Society (Pugliese *et al.*, 1989).

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Methods

Preparation of slices and superfusion apparatus

Hippocampal slices were prepared as previously described (Corradetti *et al.*, 1983). Charles River male Wistar rats, 150–200 g body weight, were killed by decapitation and their hippocampi rapidly dissected in 0°C oxygenated (95% O_2/5% CO_2) artificial cerebrospinal fluid (aCSF) of the following composition (mM): NaCl 124, KCl 3.33, KH_2PO_4 1.25, $MgSO_4$ 2, $CaCl_2$ 2, $NaHCO_3$ 25, D-glucose 10. Slices (400 μm thick) were cut by a McIlwain tissue chopper and kept in oxygenated aCSF for at least 1 h at room temperature. A single slice was then placed on a nylon mesh, completely submerged in a small chamber and superfused with oxygenated aCSF (32–33°C) at a constant flow rate of 2–3 ml min^{-1}. Drugs were applied via a three-way tap with complete exchange of the chamber volume in < 1 min.

Stimulation and recording technique

Test pulses (80 μs, 0.1 Hz) were delivered through bipolar nichrome electrodes positioned in the stratum radiatum. Long-term potentiation (LTP) was produced by 2 trains (100 Hz, 0.4 s, 5 min interval) of submaximal stimulation strength. LTP was considered to occur when the initial slope of the field e.p.s.p. was increased by more than 30%. Evoked orthodromic potentials (chiefly comprising dendritic responses) were extracellularly recorded with a 3 M NaCl-filled microelectrode (1–5 MΩ) placed in the CA1 region of the stratum radiatum. Responses were amplified (Neurolog NL 104, Digitimer Ltd), digitized, averaged (3–6 responses) and

stored on floppy disks for later analysis (DATA 6000, Analogic). Slices were allowed to equilibrate for at least 30 min while their electrical responses were continuously monitored. If these responses did not change during a subsequent 15 min period, drugs were applied via the superfusion system for 10–15 min.

Measurement of recorded potentials

To avoid contamination of dendritic field e.p.s.p. by positive-going population spikes, the initial slope of the field e.p.s.p. rather than their amplitude was measured. Input-output (I/O) curves were obtained by gradual increases in stimulus strength. To construct these curves the initial slope of the field e.p.s.p. was plotted against the fibre volley amplitude. I/O curves were repeated in control conditions to ensure that the first one did not condition the following curve. The slope of the linear part of the I/O curves was fitted by linear regression and the statistical significance of the changes in the slope of the I/O curves was assessed by a computer programme (Tallarida & Murray, 1981). Values given in the text are means ± s.e.mean of measurements from different experiments. Data were analysed for their statistical significance by Student's t test.

Drugs and chemicals

Oxiracetam was obtained from I.S.F. (Trezzano sul Naviglio, Italy); D-(−)-2-amino-5-phosphonopentanoic acid (AP-5) was purchased from Tocris. Both drugs were dissolved in aCSF and applied by superfusion.

Results

The results described in this paper were obtained by extracellular recording of dendritic e.p.s.p. in 49 slices prepared from 41 rats. Bath application of oxiracetam (1 μM) increased the initial slope and the amplitude of the dendritic field e.p.s.p. in 14 out of 19 slices (Figure 1a). The average increase in the initial slope was 70 ± 11% (n = 14). After 45 min of washout of oxiracetam a 43 ± 11% increase was still present. The effect started within 5 min from the beginning of the drug superfusion and reached its maximum within 10 min. Figure 1b shows that application of 1 μM oxiracetam significantly ($P < 0.05$) changed the slope of the I/O curve which became steeper and had a greater maximal response. Conversely, as shown in Figure 1c, there was no difference between the curves relating the stimulation intensity to the amplitude of the afferent volley before and during bath-application of 1 μM oxiracetam. This finding would exclude the possibility that oxiracetam increased the number of presynaptic fibres excited by electrical stimulation. Figure 2 shows the concentration-effect relation obtained by plotting increasing concentrations of oxiracetam against the % increase in the initial slope of the field e.p.s.p. after 10 min of drug application. The maximum effect was observed with 1 μM oxiracetam.

The duration of the effect of oxiracetam (longer than 90 min) is reminiscent of the LTP induced by high frequency stimulation of the stratum radiatum. Experiments were there-

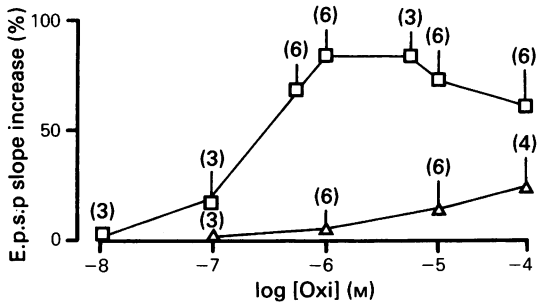

Figure 2 The effect of oxiracetam (Oxi) was concentration-dependent and antagonized by D-(−)-2-amino-5-phosphonopentanoic acid (AP-5). Cumulative concentration-response curves were constructed by adding increasing concentrations of oxiracetam to superfusion fluid in the absence (□) or presence (△) of AP-5 (50 μM). Data shown are from 3–6 experiments on different slices. Vertical lines indicate s.e.mean (number in parentheses) for each point. All points of the curve in presence of AP-5 are statistically different ($P < 0.05$) from the corresponding points of the control curve.

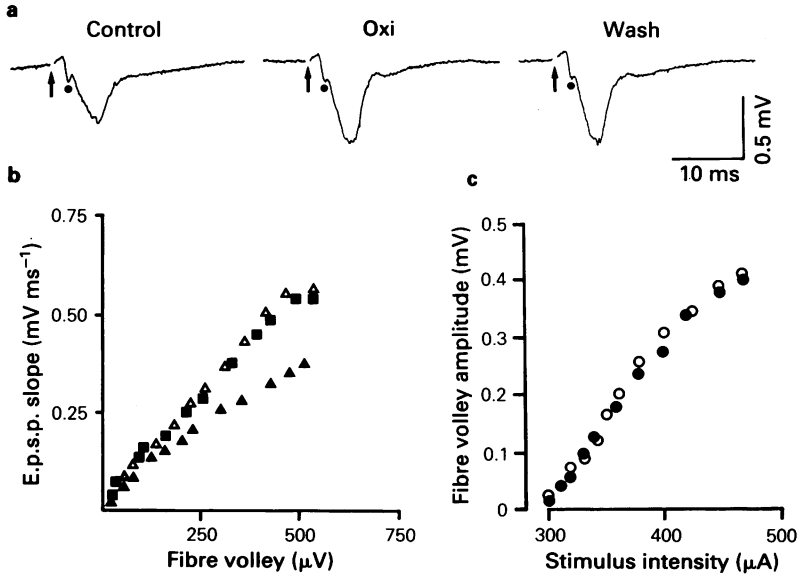

Figure 1 Oxiracetam increases evoked field e.p.s.p. in the CA1 dendritic region. (a) Stimulation (arrow) of the stratum radiatum in control conditions evoked a negative going potential interpreted as field e.p.s.p. (left trace). Superfusion with 1 μM oxiracetam (Oxi) for 10 min increases the initial slope and amplitude of the field e.p.s.p. (middle trace). These increases persisted after a 45 min wash (right trace). Note that the afferent volley (dots) is not changed by oxiracetam. (b) Input-output (I/O) curves constructed by changing stimulus strength in the presence of 1 μM oxiracetam (10 min; ■) and after 45 min washing (△) are significantly ($P < 0.05$) steeper than those obtained under control conditions (▲). Note the augmentation of the maximal response produced by oxiracetam. (c) The relationship between stimulus strength and volley amplitude obtained in control conditions (●) is not modified by 1 μM oxiracetam (○). The symbols in (b) and (c) are representative of 5 similar experiments.

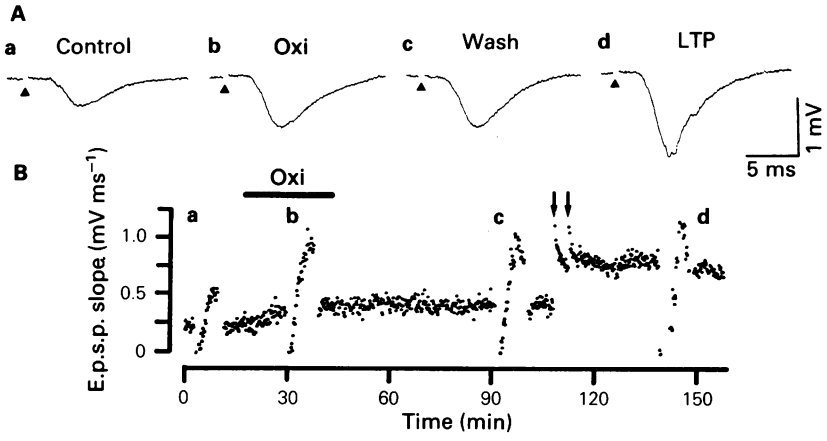

Figure 3 Effect of high frequency stimulation of the stratum radiatum after wash of oxiracetam. (A) Oxiracetam (Oxi, 1 μM) increased evoked e.p.s.p. The effect persisted after wash, long-term potentiation (LTP) could be induced by high frequency stimulation. Traces are averages of 6 evoked responses taken at times indicated by corresponding letters in (B). (▲) indicates time of stimulus. (B) Each point of the graph represents the initial slope of field e.p.s.p. elicited by test pulses (370 μA every 10 s). In addition, at representative times during the experiment, the test pulse was stopped and input-output curves were constructed by varying stimulation strength between 300 and 600 μA. These curves appear as sharp deflections of the points inserted in the trend of the test pulses. Oxiracetam (1 μM) significantly ($P < 0.05$) increased the slope of the field e.p.s.p. evoked by test pulses, significantly ($P < 0.05$) shifted the input-output curve (not appreciable by eye, but see Figure 1) and increased the maximal response. These potentiations persisted during a 1 h washing. Two trains (arrows) of high frequency stimulation (100 Hz, 0.4 s, 470 μA, 5 min apart) were followed by LTP of the response to test pulses and a further, significant ($P < 0.05$), shift of the input-output curve. The maximal response after the high frequency train was not increased in this or similar experiments.

Table 1 Percentage increase in field excitatory postsynaptic potential (e.p.s.p.) initial slope in hippocampal slices after long-term potentiation (LTP) induction and oxiracetam applications

Pretreatment	(n)	*Oxiracetam* (1 μM)	*Wash* (45 min)	*LTP* (60 min)
None	(6)	71 ± 13*a	55 ± 29*	105 ± 37*
None	(7)	—	—	99 ± 19*
LTP	(5)	27 ± 19*b	12 ± 17	—

Values are % increases over pretreatment responses. Measurements were done on 4 averaged potentials taken 10 min after the beginning of superfusion with oxiracetam. LTP pretreatment values were taken 60 min after the high frequency stimulation. Data are expressed as mean ± s.e.mean from given number (*n*) of experiments. * Denotes values significantly different from pretreatment values ($P < 0.05$, paired *t* test). [b] Denotes value significantly different from [a] ($P < 0.05$, unpaired *t* test).

fore performed to investigate whether the persistent enhancement of neurotransmission brought about by oxiracetam could influence LTP magnitude and/or development.

In the first series of experiments ($n = 6$), LTP was induced after oxiracetam (1 μM) had been applied and then washed out for 30–60 min. As shown in Figure 3, oxiracetam (1 μM) initially produced an increase in the slope and amplitude of evoked field e.p.s.p. Such an increase persisted after 45 min wash. High frequency electrical stimulation (normally used to elicit LTP) induced a comparatively small increase in the amplitude and initial slope of the field e.p.s.p. (see also Table 1). In Figure 3b the time course of the change in the initial slope is shown together with the I/O curves (constructed in control aCSF and during oxiracetam or LTP). Note that the I/O curves obtained in the presence of oxiracetam (1 μM) or 60 min after washout were significantly steeper ($P < 0.05$) and reached a greater ($P < 0.05$) maximal response than the control one. High frequency stimulation was followed by a

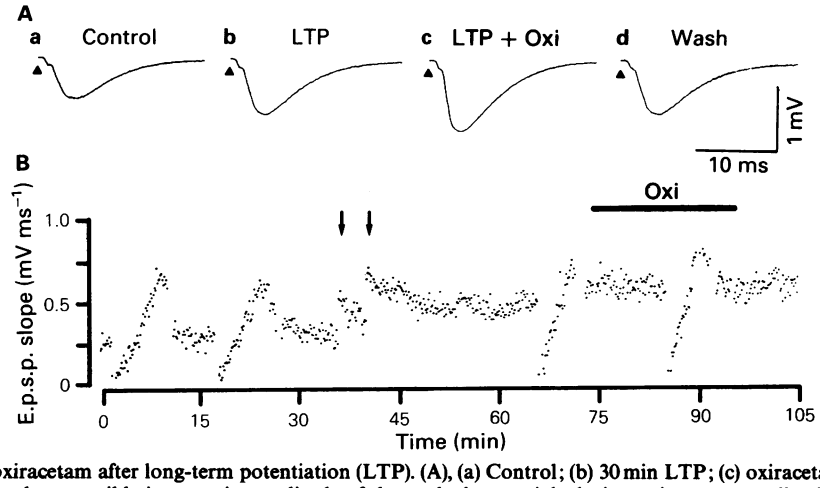

Figure 4 Effect of oxiracetam after long-term potentiation (LTP). (A), (a) Control; (b) 30 min LTP; (c) oxiracetam (Oxi) 1 μM, 10 min; (d) 30 min wash. Note the reversible increase in amplitude of the evoked potentials during oxiracetam application. (▲) Indicates time of stimulus. Traces are averages of 6 evoked responses. (B) Time-course of a representative experiment similar to that shown in (A). Each point of the graph represents the initial slope of field e.p.s.p. elicited by test pulses (550 μA every 10 s). In addition input-output curves constructed by varying stimulation strength between 400 and 900 μA are shown. Two trains (arrows) of high frequency stimulation (100 Hz, 0.4 s, 670 μA, 5 min apart) were followed by LTP of the response to test pulses and a significant ($P < 0.05$) shift of the input-output curve. Oxiracetam (1 μM) had no significant effects on the slope of the field e.p.s.p. evoked by test pulses and on the input-output curve.

further, albeit moderate increase in initial slope and amplitude of the test response. The I/O curve was significantly ($P < 0.05$) steeper than the one obtained in the presence of oxiracetam, although the maximal response did not change significantly.

Figure 4 shows the results ($n = 5$) obtained when LTP was induced before adding oxiracetam (1 μM). As expected (see Figure 4A) high frequency stimulation induced a long-lasting increase in amplitude and the initial slope of the test response, i.e. an LTP. Oxiracetam (1 μM) produced a further increase in both parameters. The effect of oxiracetam disappeared within 45 min of washout, with a return to the values previously obtained by high-frequency stimulation. In a similar experiment (Figure 4B), I/O curves were constructed before, 30 min after LTP induction, and during oxiracetam (1 μM) application. The I/O curve after LTP was significantly ($P < 0.05$) steeper than in control conditions. The effect of oxiracetam (1 μM) on the slope and maximal response of the I/O curve was small and not statistically significant ($P > 0.1$). Table 1 summarizes the results of the experiments investigating the interactions between oxiracetam and LTP. It appears that, in spite of the increase in the initial slope of the field e.p.s.p. obtained by electrical stimulation 45 min after washout of 1 μM oxiracetam, the magnitude of the LTP did not significantly differ either from that produced by the same high frequency stimulation in untreated slices or from the increase in initial slope produced by oxiracetam application. Consistently, oxiracetam had a small, yet reversible, effect once LTP was established.

In view of the similarities which appeared to exist between the effect of oxiracetam and the electrically induced LTP, we investigated whether AP-5, which has been demonstrated to block electrically evoked LTP (Collingridge *et al.*, 1983) also affected the action of oxiracetam. Figure 5a shows that 15 min after the application of 50 μM AP-5, a concentration which under our conditions blocked LTP ($+5 \pm 5\%$; $n = 3$) completely prevented the increase in field e.p.s.p. brought about by 1 μM oxiracetam ($+3 \pm 7\%$; $n = 8$). The antagonism of the effect of oxiracetam by AP-5 was also demonstrated by the identical I/O curves obtained during the application of oxiracetam (1 μM) and AP-5 (50 μM) with those under control conditions (Figure 5b). Finally, the concentration-effect relation between increasing concentrations of oxiracetam and field e.p.s.p. and the initial slope in the presence of AP-5 (50 μM; $n = 5$) is shown in Figure 2. It can be seen that the curve was shifted to the right and the effect of oxiracetam was strongly reduced.

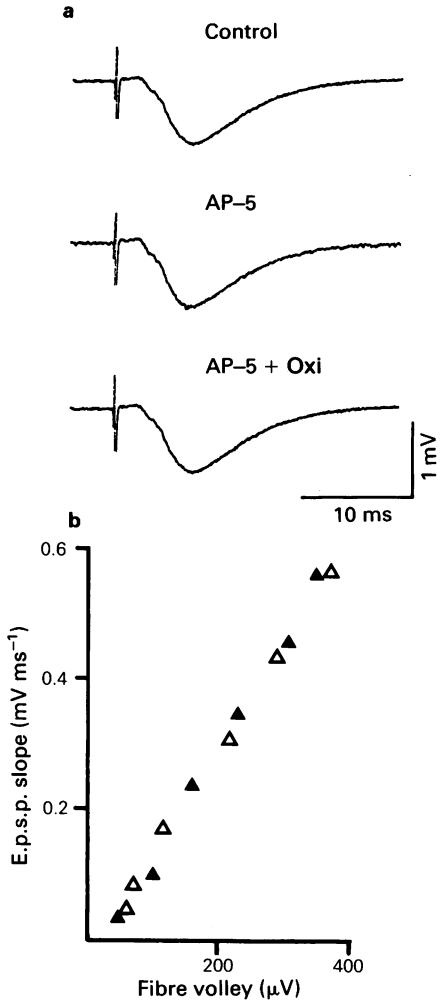

Figure 5 D-(−)-2-Amino-5-phosphonopentanoic acid (AP-5) prevents the effect of oxiracetam. (a) Stimulation (rapid biphasic artifact) of the stratum radiatum evoked a field e.p.s.p. in control aCSF (top trace). Addition of AP-5 (50 μM, 15 min) did not change the evoked response. In the presence of AP-5, oxiracetam (Oxi, 1 μM, 10 min) had no effect (bottom trace). Traces are averages of 4 evoked responses. (b) Input-output curves constructed in control aCSF (▲) and during (△) application of oxiracetam (1 μM) in the presence of AP-5 (50 μM) were identical. (a) and (b) were from the same experiment.

Discussion

Our experiments demonstrate that, in rat hippocampal slices, superfusion of oxiracetam brought about a long-lasting enhancement of neurotransmission at the synapses connecting the Schaffer collateral-commissural fibres with CA1 hippocampal pyramidal cells. This effect was concentration-dependent and was exerted at concentrations which are usually achieved in the hippocampus *in vivo* after oral administration of oxiracetam (Ponzio *et al.*, 1988). In the present study an apparent reduction in the effect elicited by concentrations higher than 5 μM was seen. According to Olpe *et al.* (1986) 1 mM oxiracetam is ineffective on the evoked responses in CA1. Since other *in vitro* studies with similar compounds (Satoh *et al.*, 1986; 1988) and behavioural work (Cumin *et al.*, 1982) showed a bell-shaped dose-effect relation, our findings may indicate the need to avoid unduly large concentrations of nootropics as their actions tend to dissipate following high doses.

The lack of changes in presynaptic afferent volley during oxiracetam application (Figure 1c) indicates that the enhancement of neurotransmission produced by oxiracetam was not due to an increase in the number of presynaptic fibres excited by the electrical stimulation. In our experiments we also demonstrated that the increase in field e.p.s.p. initial slope and amplitude induced by oxiracetam is blocked by AP-5, a selective antagonist of the NMDA subtype of excitatory amino acid receptors (Davies *et al.*, 1981). This observation suggests that NMDA-receptor activation is a step in the sequence of events mediating the action of oxiracetam. One may speculate on the mechanism(s) responsible for such an activation of the NMDA-receptor. Any postsynaptic agonist activity of oxiracetam on glutamate receptors, such as that suggested for piracetam on the basis of binding studies (Bering & Muller, 1985), appears unlikely. In fact such an agonist activity should lead to sustained depolarization of cells during drug superfusion and, hence, to a reduction in the field e.p.s.p. (Collingridge *et al.*, 1983). On the other hand, oxiracetam might decrease via a glycine-like mechanism (Mayer *et al.*, 1989) desensitization of NMDA-receptors possibly activated by glutamate and/or aspartate at the pre- and/or postsynaptic level. Nevertheless, the slow onset of the response to oxiracetam and its persistence after its removal, is not easily compatible with this hypothesis and perhaps lends support to the view that oxiracetam may accumulate intracellularly. Should this be the case, oxiracetam, through yet unknown mechanism(s), might enhance the response to the putative transmitters such as glutamate and/or aspartate (Corradetti *et al.*, 1983). Oxiracetam might also promote the release of other endogenous, still unidentified, neurotransmitters or modulators such as the mast cell degranulating-like peptide which has been suggested to be involved in LTP (Cherubini *et al.*, 1987). Interestingly,

the action of this peptide is also prevented by AP-5 in the CA1 region of the hippocampus.

High frequency stimulation has repeatedly been shown to induce an AP-5-sensitive LTP (see in Teyler & DiScenna, 1987; Bliss & Lynch, 1988) associated with a persistent increase in the release of aspartate and glutamate (Bliss *et al.*, 1986; but see Anikszteljn *et al.*, 1989). In fact, the AP-5 sensitivity of the effect of oxiracetam together with its partial occlusion (see Table 1) of the electrically-produced LTP suggests that NMDA-receptors were involved in both phenomena.

In spite of the fact that differences in the action of piracetam and related nootropic drugs in many behavioural tests are seemingly related only to the doses used (Cumin *et al.*, 1982; Schindler *et al.*, 1984), the few data available for the electrophysiological effects of these drugs on the hippocampus suggest a different site and/or mechanism of action for each compound. Piracetam, for instance, has been found to increase reversibly the amplitude of the population spike, without any effect on the dentritic field e.p.s.p. (Olpe & Lynch, 1982) or on LTP in the CA1 region (Olpe & Lynch, 1982; Satoh *et al.*, 1988). On the other hand, both piracetam and aniracetam have been shown to augment the magnitude of LTP produced in the CA3 region of the guinea-pig hippocampus, without changing field potentials induced by single pulse stimulation (Satoh *et al.*, 1986; 1988). The action of oxiracetam appears to differ from that of piracetam and aniracetam since it induced a persistent, AP-5 sensitive and LTP-like increase in neurotransmission efficacy in the CA1 region. Since NMDA-receptor antagonists prevent LTP induction (Collingridge *et al.*, 1983; Harris *et al.*, 1984) and the acquisition of conditioned responses (Morris *et al.*, 1986), it appears that NMDA-receptors play an important role in learning mechanisms, including LTP. Therefore, the enhancement of hippocampal synaptic transmission by oxiracetam, demonstrated in our experiments, indicates that NMDA-receptor activation is probably an important characteristic of the nootropic properties of oxiracetam.

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References

ANIKSZTEJN, L., ROISIN, M.P., AMSELLEM, R. & BEN-ARI, Y. (1989). Long-term potentiation in the hippocampus of the anaesthetized rat is not associated with a sustained enhanced release of endogenous excitatory amino acids. *Neuroscience*, **28**, 387–392.

BANFI, S. & DORIGOTTI, L. (1986). Experimental behavioral studies with oxiracetam on different types of chronic cerebral impairment. *Clin. Neuropharmacol.*, **9** Suppl. 3, S19–S26.

BANFI, S., DORIGOTTI, L., ABBRACCHIO, M.P., BALDUINI, W., COEN, E., RAGUSA, C. & CATTABENI, F. (1984). Methylazoxymethanol microencephaly in rats: neurochemical characterization and behavioral studies with the nootropic oxiracetam. *Pharmacol. Res. Commun.*, **16**, 67–83.

BERING, B. & MULLER, W.E. (1985). Interaction of piracetam with several neurotransmitter receptors in the central nervous system. *Arzneim. Forsch.*, **35**, 1350–1352.

BLISS, T.V.P., DOUGLAS, R.M., ERRINGTON, M.L. & LYNCH, M.A. (1986). Correlation between long-term potentiation and release of endogenous amino acids from dentate gyrus of anesthetized rats. *J. Physiol.*, **377**, 391–408.

BLISS, T.V.P. & LYNCH, M.A. (1988). Long-term potentiation of synaptic transmission in the hippocampus: properties and mechanism. In *Long-Term Potentiation: From Biophysics to Behavior*, ed. Landfield, P.W. & Deadwyler, S.A. pp. 3–72. New York: Alan Liss, Inc.

BROWN, T.H., CHAPMAN, P.F., KAIRIS, E.W. & KEENAN, C.L. (1988). Long-term synaptic potentiation. *Science*, **242**, 724–728.

CHERUBINI, E., BEN-ARI, Y., GHO, M., BIDARD, J.N. & LAZDUNSKI, M. (1987). Long-term potentiation of synaptic transmission in the hippocampus induced by a bee venom peptide. *Nature*, **328**, 70–73.

COLLINGRIDGE, G.L., KEHL, S.J. & McLENNAN, H. (1983). Excitatory amino acids in synaptic tràns mission in the Schaffer collateral-commissural pathway of the rat hippocampus. *J. Physiol.*, **334**, 33–46.

CORRADETTI, R., MONETI, G., MORONI, F., PEPEU, G. & WIERASZKO, A. (1983). Electrical stimulation of the stratum radiatum increases the release and neosynthesis of aspartate, glutamate and γ-aminobutyric acid in rat hippocampal slices. *J. Neurochem.*, **41**, 1518–1525.

CUMIN, R., BANDLE, E.F., GAMZU, E. & HAEFELY, W.E. (1982). Effects of the novel compound aniracetam (Ro 13-5057) upon impaired learning and memory in rodents. *Psychopharmacol.*, **78**, 104–111.

DAVIES, J., EVANS, R.H., JONES, A.W. & WATKINS, J.C. (1981). 2-amino-phosphonovalerate (2APV), a potent and selective antagonist of amino acid-induced and synaptic excitation. *Neurosci. Lett.*, **21**, 77–81.

GAINOTTI, G., BENEDETTI, N., CALTAGIRONE, C. & NOCENTINI, U. (1986). Cognitive improvement in clinical trials with nootropic drugs: when can it be expected and how to clarify its meaning. *Clin. Neuropharmacol.*, **9** Suppl. 3, S65–S69.

GIAQUINTO, S., NOLFE, G. & VITALI, S. (1986). EEG changes induced by oxiracetam on diazepam-medicated volunteers. *Clin. Neuropharmacol.*, **9** Suppl. 3, S79-S84.

GIURGEA, C. & SALAMA, M. (1977). Nootropic drugs. *Prog. Neuropsychopharmacol.*, **1**, 235–247.

HARRIS, E.W., GANONG, A.H. & COTMAN, C.W. (1984). Long-term potentiation in the hippocampus involves activation of N-methyl-D-aspartate receptors. *Brain Res.*, **323**, 132–137.

MAYER, M.L., VYCLICKY, L. Jr. & CLEMENTS, J. (1989). Regulation of NMDA receptor densensitization in mouse hippocampal neurons by glycine. *Nature*, **338**, 425–427.

MOOS, W.H., DAVIS, R.E., SCHWARZ, R.D. & GAMZU, E.R. (1988). Cognition activators. *Med. Res. Rev.*, **8**, 353–391.

MORRIS, R.G.M., ANDERSON, E., LYNCH, G.S. & BAUDRY, M. (1986). Selective impairment of learning and blockade of long-term potentiation by an N-methyl-D-aspartate receptors antagonist AP5. *Nature*, **319**, 774–776.

NICOLL, R.A., KAUER, J.A. & MALENKA, R.C. (1988). The current excitement in long-term potentiation. *Neuron*, **1**, 97–103.

OLPE, H.R. & LYNCH, G.S. (1982). The action of piracetam on the electrical activity of the hippocampal slice preparation: a field potential analysis. *Eur. J. Pharmacol.*, **80**, 415–419.

OLPE, H.R., POZZA, M.F., JONES, R.S.G. & HAAS, H.L. (1986). Comparative electrophysiological investigation on oxiracetam and piracetam. *Clin. Neuropharmacol.*, **9** Suppl. 3, S48–S55.

PEPEU, G. & SPIGNOLI, G. (1989). Neurochemical actions of nootropic drugs. In *Alzheimer's Disease*. Proceedings of the fifth meeting of the international study group of the pharmacology of memory disorders associated with aging. Zurich, Switzerland. (Compilers' Wurtman, R.J., Corkin, S., Growdon, J.H. & Ritter-Walker, E.), pp. 387–398.

PONZIO, F., POZZI, O., BANFI, L. & DORIGOTTI, L. (1988). Experimental evidences of oxiracetam direct activity on memory mechanisms. *Psychopharmacol.*, **96** Suppl. 71.

PUGLIESE, A.M., CORRADETTI, R. & PEPEU, G. (1989). Effect of the cognition enhancing agent oxiracetam on electrical activity of hippocampal slices. *Br. J. Pharmacol.*, **96**, 80P.

SATOH, M., ISHIHARA, K., IWANA, T. & TAKAGI, H. (1986). Aniracetam augments, and midazolam inhibits, the long-term potentiation in guinea pig hippocampal slices. *Neurosci. Lett.*, **68**, 216–220.

SATOH, M., ISHIHARA, K. & KATSUKI, H. (1988). Different susceptibilities of long-term potentiations in CA3 and CA1 regions of guinea pig hippocampal slices to nootropic drugs. *Neurosci. Lett.*, **93**, 236–241.

SCHINDLER, U., RUSH, D.K. & FIELDING, S. (1984). Nootropic drugs: animal models for studying effects on cognition. *Drug. Dev. Res.*, **4**, 567–576.

SPIGNOLI, G. & PEPEU, G. (1986). Oxiracetam prevents electroshock-induced decrease in brain acetylcholine and amnesia. *Eur. J. Pharmacol.*, **126**, 253–257.

SPIGNOLI, G. & PEPEU, G. (1987). Interactions between oxiracetam, aniracetam and scopolamine on behaviour and brain acetylcholine. *Pharmacol. Biochem. Behav.*, **27**, 491–495.

TALLARIDA, R.J. & MURRAY, R.B. (1981). In *Manual Pharmacologic Calculations*, ed. Tallarida, R.J. & Murray, R.B., pp. 11–13. New York: Springer-Verlag.

TEYLER, T.J. & DiSCENNA, P. (1987). Long-term potentiation. *Ann. Rev. Neurosci.*, **10**, 131–161.

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